Ligands, Receptor Models, and Evolution

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With the coming of age of molecular neurobiology, many components of signaling systems in the brain are now characterized to some extent at the molecular level, and a reductionism approach towards a more complete synthesis of neurofunction now seems possible. In the case of neurotransmitter receptors the pace at which their gene sequences and derived primary structures have been acquired has been astounding. Several surprises have emerged from this work, the most notable of which is that only a few structural classes of neurotransmitter receptors exist. Two major superfamilies have been identified, the ligand-gated ion-channel (LGIC) receptors,[1] and the G-protein coupled receptors (GPCR).[2] They mediate the fast "all or nothing" and the slow-graded modes of chemical communication, respectively. Importantly, they encompass the majority of the receptor neuropharmacology that has accumulated to date.

In the case of the LGIC receptors, the structural and conformational requirements for agonist and antagonist binding to the receptors of each of them separately have been extensively covered in the literature. In this paper a more unified pharmacophore is proposed for the LGIC receptors.

LIGAND-GATED ION-CHANNEL AGONISTS

FIGURE 1 shows a collection of agonist structures for the different types of LGIC receptors. In rows from top to bottom are the neurotransmitters, semirigid analogues with restricted rotatable dihedral bonds, and the almost totally rigid agonist analogues. Several comparisons are possible in the context of the superfamily. The common features for binding to the LGIC receptors can be identified as (1) an amine group, (2) a π-bonded system, (3) an sp^2 electronegative atom within the π-system, and (4) small size (i.e. < 15 nonhydrogen atoms). The unified receptor pharmacophore model incorporates these conserved features. The broad similarity of agonists is strikingly demonstrated by comparison with the almost totally rigid analogues cytisine and THIP (4,5,6,7-tetrahydroisoxazolo[5,4-c]pyridin-3-ol), which are agonists of the evolutionary distantly related nicotinic acetylcholine and $GABA_A$ receptors, respectively.

For the amine group, this can vary from a primary through to a quaternary group, even for a given receptor type (see nAChR column in FIG. 1). Acetylcholine is an informative molecule because it establishes that the role for the conserved nitrogen center is to provide a positively charged group for interaction with the receptor site.

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The π-bonded system part of the molecule can also vary, both in shape and electronic properties, even for a given receptor (e.g., the pyridyl ring in nicotine instead of the acetyl group in acetylcholine). Nonetheless, it displays several unusual and interesting features that are conserved, which may be important for recognition. It is rigid-coplanar and contains an electronegative atom center that is capable of forming a hydrogen bonding interaction. Of particular note, the electronegative center forms a delocalizable local dipole over the π-system.[3]

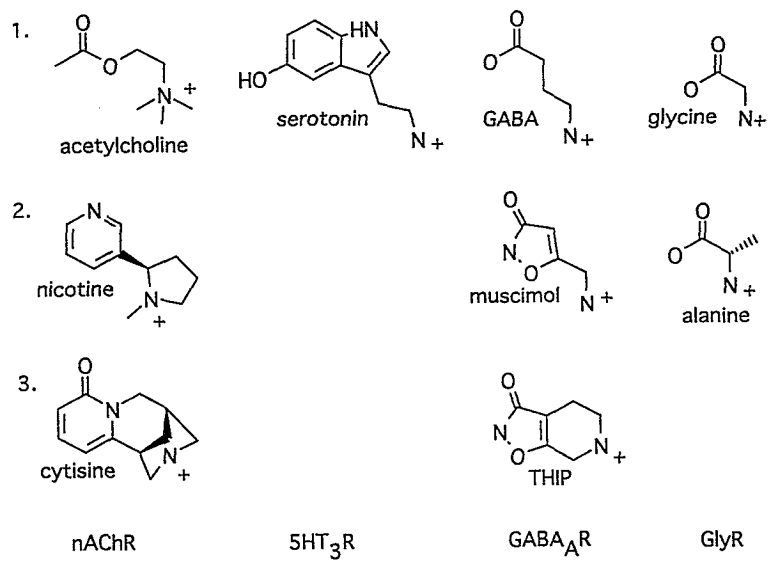

FIGURE 1. Comparison of ligand-gated ion-channel agonist structures. Agonists of the nicotinic acetylcholine-R (nAChR), $5HT_3$-R ($5H_3R$), γ-amino butyric $acid_A$-R ($GABA_A$ R), and glycine-R (GlyR) are shown. Row 1, endogenous neurotransmitters; row 2, semirigid analogues; row 3, almost totally rigid analogues. The symbol (+) highlights the positively charged amine group. Note that none of the endogenous neurotransmitter molecules contains a chiral center.

SPECIFICITY OF RECEPTOR RECOGNITION

Features leading to specific recognition can be deduced by looking for consistent differences in the ligands for the various types of receptor. However, this is a less sure exercise than identification of the common features. Differences proposed as having a role in specific recognition are:

Position and types of polar atoms within the π-system region. For $5HT_3$-R as compared to the nicotinic acetylcholine-R there is an apparent switch from a requirement for a hydrogen bond donor to a hydrogen bond acceptor.

Distance between the electronegative center of the π-system and the positive pole. This is apparent for glycine-R (3.5 Å) as compared to the $GABA_A$-R (~5.5 Å).

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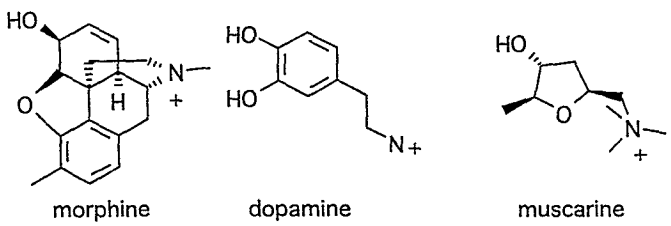

morphine dopamine muscarine

FIGURE 2. Examples of monoamine G-protein coupled receptor agonist structures. Common features are (1) the positively charged amine group and (2) the occurrence of a ring hydroxyl at position that is six bond lengths away from the amine group.

Hydrophobic and polar-charged nature of the π-system region. This places the cationic channel and anionic channel receptors into two separate groupings.

Size of the π-system. This might be of secondary importance to (1) above in achieving specific recognition at the $5HT_3$-R and nicotinic acetylcholine-R.

Of course differences in the ligands must also reflect differences in the receptors, but in the context of a conserved binding site for the superfamily.

OTHER RECEPTOR SUPERFAMILIES

A comparison of LGIC receptor agonists with those of the G-protein coupled receptors is useful to assess the significance of the proposed common features of the LGIC agonists. This is possible because some of the endogenous neurotransmitters act on receptors of both classes. This is the case at least for acetylcholine and serotonin. Of the structures shown, muscarine (FIG. 2) is an informative molecule because it establishes that a π-bonded system is not essential for agonist interaction at G-protein coupled receptors.

Initially it was expected that glutamate receptor subunits would also fall into the nicotinic-containing LGIC superfamily. Instead, based on analysis of multiple aligned sequences it can be seen that no apparent homology exists between the classes. Indeed, the extracellular domain of the glutamate receptors is more similar to a family of bacterial amino acid binding proteins than it is to any LGIC receptor.[4,5] This raises the possibility that in the LGIC superfamily the amine has to be an isolated group and cannot be functionalized such as in the form of an amino acid moiety.

LGIC ANTAGONISTS AND MODULATORS

In some antagonist structures the skeleton of their corresponding agonists can be identified (FIG. 3). For the nicotinic acetylcholine-R, the natural product methyllycaconitine (MLA) is a clear example.[6] This is of interest because the parent structure aconitine is known to act on voltage-gated sodium channels, but has no reported activity at nACh receptors. With the ester linkage in MLA a framework is introduced that can be fitted to acetylcholine. For GABA, it has already been proposed that bicucullin contains a region with the electronic properties resembling the carboxylate group of the endogenous transmitter.[7] This correspondence can also be extended to

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the $GABA_A$-R modulators flumazenil and ethyl-β-carboline-3-carboxylate. When such compounds are overlayed using the three site-points present in the agonist unified pharmacophore model, an impression of structural conservation outside the agonist binding region is obtained (see FIG. 4).

UNIFIED LGIC PHARMACOPHORE MODEL

FIGURE 5 shows a unified LGIC receptor pharmacophore model. The agonist site comprises the positive pole and the oriented local dipole with the electronegative

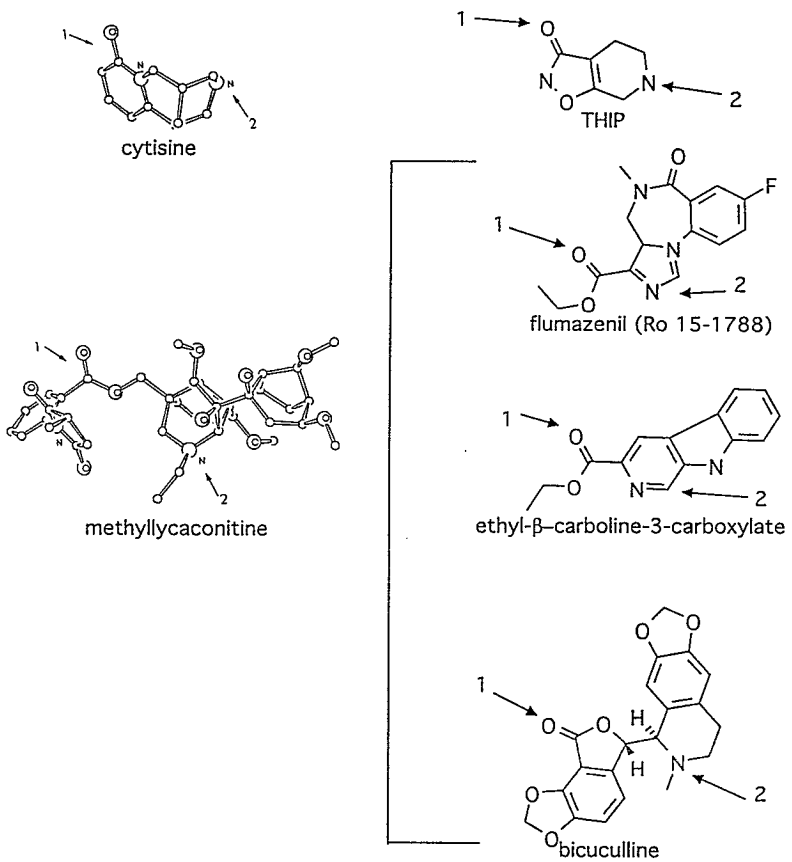

FIGURE 3. Comparison of ligand-gated ion-channel antagonists, modulators, and agonists. Methyllycaconitine is a competitive antagonist of neuronal nicotinic acetylcholine-R;[6] cytisine is a potent agonist of nicotinic acetylcholine-R; bicuculline is a competitive antagonist of $GABA_A$-R; ethyl-*b*-carboline-3-carboxylate is an inverse agonist of $GABA_A$-R;[7] flumazenil is a positive modulator of $GABA_A$-R: THIP is a potent agonist of $GABA_A$-R.

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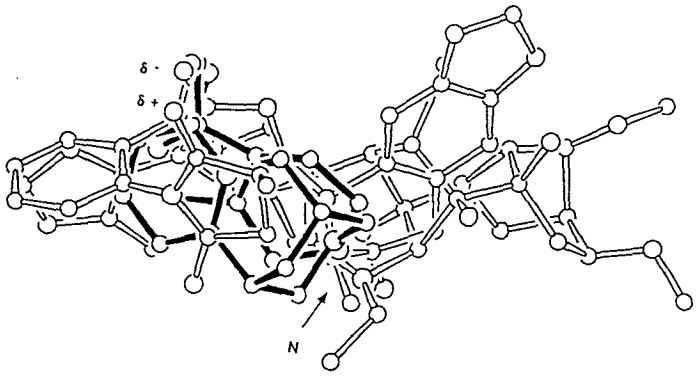

FIGURE 4. Overlay of dissimilar ligand-gated ion-channel antagonist structures. The rigid agonists cytisine and THIP (*solid bonds*) and the antagonists methyllycaconitine and bicuculline are shown. Superpositioning was done using three points: the nitrogen atom of the positive pole and the electronegative and electropositive sp^2 atom centers of the oriented dipole. The agonist-antagonist pairs THIP-bicuculline and cytisine-methyllycaconitine were first superimposed. These pairs were then overlaid by superpositioning of the agonists THIP and cytisine. THIP, 4,5,6,7-tetrahydroisoxazolo[5,4-c] pyridin-3-ol.

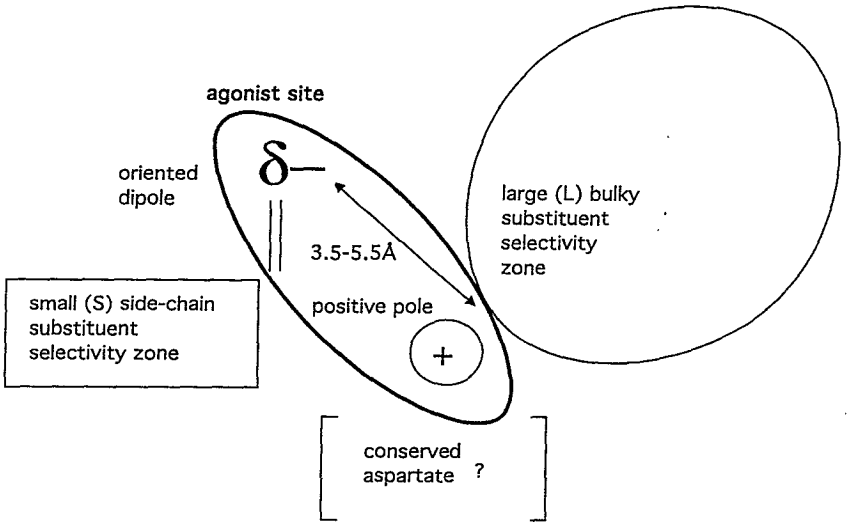

FIGURE 5. Unified ligand-gated ion-channel pharmacophore model. *Symbols*: ⊕ = the positive pole; δ− = electronegative atom of the π-system. The large (L) and small (S) regions outside the agonist core are regions of the binding site that can be occupied by antagonists.

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atom center available for hydrogen bonding. Outside the agonist core two regions are proposed as being occupied by antagonist molecules. The large region (L) corresponds to the aconitine portion of methyllycaconitine, whereas the smaller region (S) is occupied by its *N*-phenylsuccinimide moiety. In the case of the benzodiazepine modulators only the large region is occupied.

Inasmuch as the *N*-phenylsuccinimide moiety of methyllycaconitine is topologically equivalent to the reactive group in ligands that label the α-subunit cysteines 192–193 of the *Torpedo* nicotinic acetylcholine-R, it can be expected that this moiety contacts the surface area of the binding cavity. In contrast, the oxygen-rich large bulk

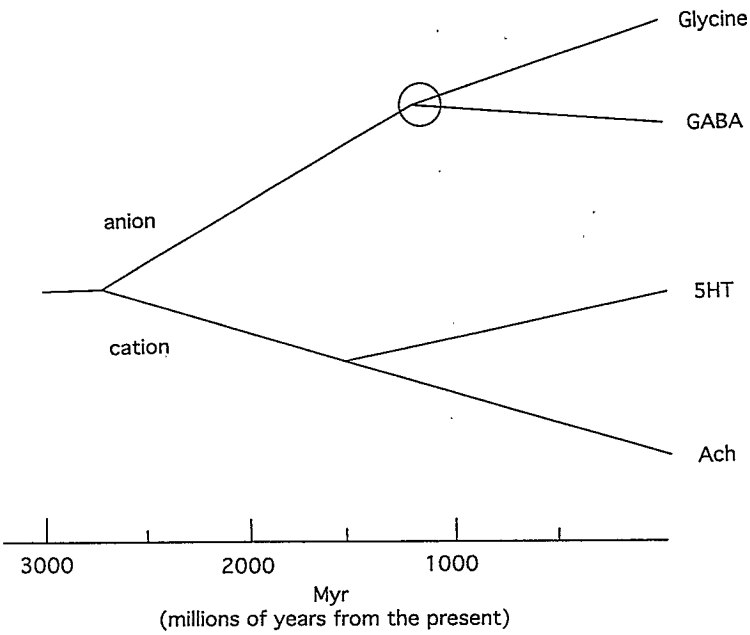

FIGURE 6. Ligand-gated ion-channel receptor evolution. The tree was constructed using 106 nucleic acid sequences.[8] The tree presented shows only branch points for early events in receptor evolution. The circling of the bifurcation point for the GABA and glycine branches signifies that they are not sister branches.

of the aconitine portion of methyllycaconitine could be facing out towards the solvent. Interaction with parts of neighboring subunits could then be possible, as is observed with *d*-tubocurarine, a competitive antagonist of nicotinic acetylcholine-R.[8]

From the unified pharmacophore model a hypothetical recognition pathway model is proposed. Starting from when an agonist molecule is within 12 Å of the assumed conserved aspartate of the binding site, long-range electrostatic interaction between the negative charge of this residue and the positive pole of the agonist is sufficient to cause the agonist to be attracted towards it. On closer approach, at 6 Å, the local dipole of the agonist could become oriented in the electrostatic field of the

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aspartate residue. The steering of the ligand at this stage may assist the entry of agonist into the binding cavity. On formation of the binary complex, the overwhelming electrostatic field of the aspartate residue increases the size of the local dipole of the π-system, causing a shift in electron density over the electronegative atom. Synergism for the interaction of agonist with the receptor could be obtained by this induction step, because the electronegative atom becomes a more effective hydrogen bonding group.

RECEPTOR EVOLUTION

The evolutionary tree in FIGURE 6 shows branch points representing the early separation of different types of LGIC receptor types.[9] Under the assumption of the analysis, the initial branch point would have been at least 2500 million years ago. This would roughly exceed current estimates for the time of origin of eukaryotes. Although this is a surprisingly early start for the LGIC receptors, in the case of G-protein coupled receptors it is now reasonably well founded that bacteriorhodopsin is structurally homologous to the vertebrate rhodopsins. It is also apparent from the tree that the origins of the distinct receptor types are also ancient, occurring at some time when multicellular organisms were starting to evolve.

Given the hierarchical nature of divergent evolution, the question can be asked, How might receptor evolution have had an impact on current day pharmacological probing of receptor superfamilies? Not too surprisingly, examples have appeared in the literature where the selectivity of a ligand acting at a subset of receptors reflects evolutionary relatedness. The adrenergic pharmacological classification scheme appears to be a good example of this.[10] However, there are other examples where a particular ligand interacts with a receptor distantly related to its classical pharmacologically defined site.[11] Examination of these unusual instances, termed as leakage specificity, will no doubt allow understanding of how an isolated pairwise interaction between a group in the ligand and an amino acid side-chain in receptors can influence pharmacological specificity.

In summary, evaluating classical pharmacology in the context of a superfamily can involve comparison of ligands and construction of receptor homology models, and should be viewed in an evolutionary setting.

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